REMARKS

Applicants filed an Amendment and Response to the June 23, 2010 Final Office Action on December 23, 2010. An Examiner interview was subsequently conducted on March 21, 2011. Applicants submit herewith a Supplemental Amendment, including the amended claims discussed during the March 21, 2011 Examiner interview. The present claim listing replaces all prior versions and listing of claims in this application. The comments submitted herewith are intended to supplement, and/or be considered in conjunction with, the remarks and arguments submitted in the December 23, 2010 Response. Entry and consideration of this Amendment is respectfully requested.

Claims 1-40, 43 and 44 are pending in this application. Claim 1 has been amended, *supra*, without admission and without prejudice to Applicants' right to pursue any subject matter that may have been surrendered by this amendment in either this or other (*e.g.*, related divisional and/or other continuing) applications. Specifically, claim 1 has been amended for clarity and to include the limitations recited in claims 12-14. Claims 3-4 have been amended for clarity. Support for these amendments can be found in the specification as filed, *e.g.*, at pages 19-20. Claims 12-14 have been canceled. No new matter has been added to this application. Upon entry of this amendment, claims 1-11, 15-40, 43 and 44 will be pending and under consideration.

Examiner Interview Summary:

Applicants gratefully acknowledge the courtesy shown by Examiner Maury Audet during a telephonic interview with Applicants' representative, Peter Ludwig, on March 21, 2011. During the interview, the claim amendments set forth in the present claim listing were discussed with the Examiner. It was explained by Applicants that an important aspect of the inventive process includes addition of a flocculating agent, as described in the specification at paragraphs 32, 67, 70, 72 and 74. The Examiner acknowledged that the specification refers to the general use of "flocculating agents," but indicated that no species of this genus are listed in the disclosure. Accordingly, Applicants' attorney agreed to submit literature references demonstrating that flocculating agents are an art recognized class of materials.

Rejection under 35 U.S.C. § 102/103:

Claims 1-40, 43 and 44 remain rejected under 35 U.S.C. § 102(b) as anticipated by, or alternatively, under 25 U.S.C. § 103(a) as obvious over U.S. Patent Nos. 6,323,276 ("Horowitz I"), 6,228,934 ("Horowitz II"), 6,368,836 ("Horowitz III"), 4,968,611 ("Traussnig"), and 5,213,976 ("Blauhut"). According to the Examiner, it would have been obvious to perform the same process steps, or make routine optimizations thereof, to recover the claimed polyhydroxyalkanoate (PHA) species recited in the claims. The Examiner also cites numerous secondary references[1] which are said to teach isolation of the PHA species. This rejection is respectfully traversed.

The claims, as amended, now include important process steps for obtaining the cellular biomass slurry. In particular, the amended claims specify, *inter alia*, a step of adding a flocculating agent to the biomass suspension.

Applicants respectfully submit that the flocculation step contributes to a number of unexpected and important features of the present invention. In particular, it was surprisingly discovered that the flocculation step facilitates micro-filtration, providing enhanced rates of filtration and increased recovery of PHA from the biomass. Flocculation also allows for substantial removal of cellular waste during the extraction step of the polymer by the solvent. These advantages persist throughout the entire extraction process, allowing the use of hydrocyclones during the micro-filtration.

Without being bound by theory, it is thought that the flocculating agent forms a bridge with the cell walls of the microorganisms containing the PHA with a resulting positive charge. The aggregated species form a stable and high density flake-like structure through the flocculating agent. The stabilized flakes, which contain cells with accumulated PHA, are then easily separated from the surrounding fermented liquid culture medium containing impurities. None of these advantageous features are disclosed or could have been predicted from the methods disclosed in the prior art.

Finally, Applicants submit herewith literature references establishing that flocculating agents are well known materials in the chemical arts. As described in Gessner G. Hawley, THE CONDENSED

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[1] In the Office Action, the Examiner has cited, but not relied upon, the following references: U.S. Patent Nos. 6,410,096 (Eggink I); 5,958,480 ("EgginkII"); 5,942,597 ("Noda I"); 5,821,299 (NodaII); and 5,536,419 ("Escalona").

CHEMICAL DICTIONARY 468 (10th ed. 1981), it was well known at the time of the invention that flocculating agents include substances that induce aggregation of suspended particles in such a way that they form small clumps or tufts. Inorganic flocculants were known to include, e.g., lime, alum and ferric chloride. Polyelectrolytes were well known examples of organic flocculants. Applicants also submit ten abstracts from peer reviewed journals describing several suitable flocculating agents known at the time of the invention. Accordingly, flocculating agents represent a class of materials that were well known to those skilled in the field of chemistry.

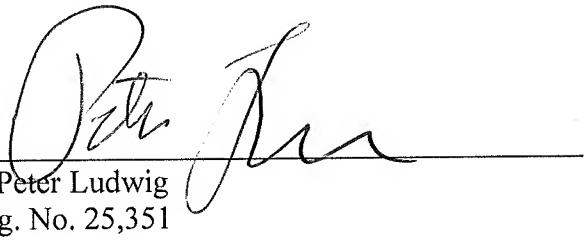
For at least the foregoing reasons, and for the reasons set forth in our December 23, 2010 Response, claims 1-40, 43 and 44 are not anticipated by and/or obvious over any of the cited references. Withdrawal of these rejections is respectfully requested.

Conclusion:

For at least the foregoing reasons, Applicants respectfully submit that each of the outstanding rejections to this application has been overcome and that the application is in condition for allowance. Accordingly, withdrawal of all rejections and allowance of the pending claims is respectfully requested. The Examiner is moreover invited to contact Applicants' undersigned representative should (s)he conclude that there are additional issues that could be readily resolved, *e.g.*, in an interview or by Examiner's Amendment. An allowance is earnestly sought.

Respectfully submitted,

Date: 5-17-11



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The Condensed Chemical Dictionary

TENTH EDITION

Revised by

GESSNER G. HAWLEY

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through an airblast. Do not confuse with flotation (q.v.).

flocculant. A substance that induces flocculation (q.v.). Flocculants are used in water purification, liquid waste treatment and other special applications. Inorganic flocculants are lime, alum, and ferric chloride; polyelectrolytes (q.v.) are examples of organic flocculants.

flocculation. The combination or aggregation of suspended colloidal particles in such a way that they form small clumps or tufts. The word is derived from this appearance. Carbon black displays a tendency to flocculate in rubber when improperly dispersed, and some clays have the same property. Oil-well drilling muds are made alkaline to prevent flocculation of their components. Flocculation can often be reversed by agitation, as the cohesive forces are relatively weak. This is not true of other forms of aggregation (coalescence and coagulation), which are irreversible.
See also agglomeration; aggregation.

flock. A light powder comprised of ground wood or cotton fibers used as an extender or filler in plastics, low-grade rubber and flooring compositions.

"Floform."[319] Trademark for carbon black used for various industrial purposes and especially for compounding with rubber.

"Flo-Gard."[177] Trademark for amorphous calcium polysilicate used as an anticaking agent for salt.

"Flogel."[266] Trademark for regular, high-density, and aluminized grades of water-resistant, nonnitroglycerin slurry explosives so formulated that a strong booster is required for their detonation.

"Flomet-Z."[329] Trademark for a fine, white, grit-free powder containing 12.5 to 14.0% zinc oxide. Used as a lubricant in powdered iron metallurgy.

flooding, chemical. See chemical flooding.

"Florex."[98] Trademark for attapulgite which has been substantially improved in adsorption efficiency by high-pressure extrusion and other special processes. Recommended where good adsorption capacity is essential. Available in various grades.

"Florisil."[98] Trademark for a highly selective adsorbent of extremely white, hard granular or powdered magnesia-silica gel (magnesium silicate).
"Florisil" TLC. Special grade for thin layer chromatography.
Uses: Preparative and analytical chromatography.

"Florite" Activated Bauxite.[98] Trademark for products produced from carefully selected domestic and imported bauxite ores. Used in the refining of petroleum oils, distillates, petrolatums, waxes and many other organic solvents; also as desiccant for gases and liquids.

"Floropryl."[123] Trademark for diisopropyl fluorophosphate.

flotation. A method of separating minerals from waste rock, or solids of different kinds from one another by agitating the pulverized mixture of solids with water, oil, and special chemicals which cause preferential wetting of solid particles of certain types by the oil, while other kinds are not wet. The unwetted particles are carried to the surface by the air bubbles and thus separated from the wetted particles. A frothing agent is also used to stabilize the bubbles in the form of a froth which can be easily separated from the body of the liquid (froth flotation). Do not confuse with floatation (q.v.).

"Flotronics."[535] Trademark for metal membrane filters of uniformly porous silver, available in distinct porosity grades from 0.2 to 5 micron maximum pore diameter. No bonding agent or fiber is used; the filters are of uniform particles of pure silver only. Used for filtration, clarification, and cold sterilization of all types of solutions.

"Flovis."[73] Trademark for a modified polyoxyethylene fatty-acid ester.
Properties: Cream to tan solid; m.p. 39–42° C; sp. gr. 1.02 (25° C); pH 5% aqueous dispersion 3–5 (25° C).
Uses: Textile and adhesive industries for stabilizing starch solutions, fluid or heavy paste, against "setting-up."

"Flowbrite."[142] Trademark for a formulation of oils used at elevated temperature for the bright flowing of electroplated tin.

flow diagram (flow sheet). A chart or line drawing used by chemical engineers to indicate successive steps in the production of a chemical, materials input and output, by-products, waste, and other relevant data.

flowers. A fine powder usually resulting from sublimation of a substance, e.g., flowers of sulfur. The term is now obsolete.

flox. A mixture of liquid fluorine (30%) and liquid oxygen (70%), designed for use as a space vehicle propellant.
Containers: Stainless steel tanks.
Hazard: Highly flammable.

"Flozan."[244] Trademark for an anhydrous soda ash product. Contains a bicarbonate of soda and a monohydrate of soda ash. White, free-flowing and will absorb approximately 30% of its weight.
Uses: Cleansing and detergent preparations.

fluid. Any material or substance that changes shape or direction uniformly in response to an external force imposed upon it. The term applies not only to liquids, but to gases and to finely divided solids. Fluids are broadly classified as Newtonian and non-Newtonian depending on their obedience to the laws

Non-Patent Literature Disclosing Flocculating Agents:

Title	Biobased polymeric flocculants for industrial effluent treatment
Author(s)	Singh, RP | Nayak, BR | Biswal, DR | Tripathy, T | Banik, K
Source	MATERIALS RESEARCH INNOVATIONS | 7 (5): 331-340 OCT 2003
Times Cited	26
Publication Year	2003
Abstract	Water is a scare commodity now. Recycling of municipal wastewater, industrial and mineral processing effluents require treatment with the inorganic or organic flocculants. Inorganic flocculants are used in large quantities, leave large amount of sludge and are very much affected by pH changes. Other polymeric flocculants are used in a very minute quantity, form large cohesive floc and are inert to pH changes. Both natural and synthetic polymers are used as flocculants. Natural polymers are biodegradable and are effective at very large dosages but are very shear stable. The synthetic polymers are highly effective flocculants at very small dosages and have high tailorability, but have poor shear stability. In the authors' laboratory, a new class of flocculants has been developed by grafting polyacrylamide branches onto polysaccharide such as guargum, hydroxypropyl guargum, starch, amylopectin and sodium alginate. These grafted polysaccharides exhibit synergistic flocculation characteristics, shear stability and controlled biodegradability. Out of all the grafted polysaccharides, amylopectin grafted polysaccharides out performs most of the commercially available flocculants. The polacrylamide branches grafted on high molecular weight branched amylopectin have larger approachability to contaminants in the industrial effluents [1, 2].
ISSN	*1432-8917*

Title	Cationic microparticle based flocculation and retention systems
Author(s)	Yan, ZG | Deng, YL
Source	CHEMICAL ENGINEERING JOURNAL | 80 (1-3): 31-36 Sp. Iss. SI DEC 1 2000
Times Cited	18
Publication Year	2000
Abstract	Two types of microparticles, cationic silica particles (CSP) and cationic polymeric microparticles (CPMP), were used as flocculants and retention aids for solid suspensions in water and pulp, respectively The solid suspensions used in this study include both positively and negatively charged precipitated calcium carbonate (PCC) and negatively charged polystyrene latex. It was found that CPMP was not an effective retention aid for positively charged PCC suspension in pulp if it was used alone. However, the retention of PCC was improved when negatively charged PCC was used. The combination of cationic microparticle systems (CPMP and CSP) with either cationically or anionically charged water-soluble polyacrylamides could significantly improve the flocculation and retention. The possible mechanisms of flocculation of solid suspensions with different dual-flocculant systems were discussed.
ISSN	*1385-8947*

Title	Characterization of an extracellular flocculating substance produced by a planktonic cyanobacterium, Anabaena sp.
Author(s)	Choi, CW | Yoo, SA | Oh, IH | Park, SH
Source	BIOTECHNOLOGY LETTERS | 20 (7): 643-646 JUL 1998
Times Cited	9
Publication Year	1998
Abstract	Two planktonic cyanobacteria, Anabaena sp. N1444 and Anabaena sp. PC-I, and a green eukaryotic alga, Scenedesmus sp,, produced extracellular flocculants. The flocculant of Anabaena PC-I, when purified, was found to be a macromolecular polysaccharide consisting of neutral sugars, uronic acids, and proteins, but not keto acids, hexosamines nor fatty acids. The flocculant bound a cationic dye, Alcian Blue, indicating it to be polyanionic. The flocculating activity was high under acidic conditions, slightly enhanced by the addition of salts and metals, and increased to about 40% upon heating at 100 degrees C for 7 min. The flocculant could flocculated various inorganic and organic compounds in solution.
ISSN	*0141-5492*

Title	Evaluation of some starch derivatives containing amide groups as flocculants
Author(s)	Khalil, MI | Aly, AA
Source	STARCH-STARKE | 53 (7): 323-329 JUL 2001
Times Cited	11
Publication Year	2001
Abstract	Three types of starch derivatives namely poly(acrylamide)-starch graft copolymer, carbamoylethylated starch, and starch carbamate were prepared and evaluated as flocculants. Native and hydrolyzed maize starches were used as parent materials for these derivatives. The flocculation was followed by monitoring transmission % and weight removal %. The different factors affecting flocculation were studied. These factors include: (a) flocculant dose, (b) pH of the flocculation medium and (c) starch derivatives type, molar mass and nitrogen content. The flocculation efficiency of the three starch derivatives follows the order: poly(acrylamide)-starch graft copolymer > carbamoylethylated starch > starch carbamate. The flocculation efficiency increases on increasing the nitrogen content and/or decreasing the molar mass of the flocculant. The flocculation efficiency of various flocculants at pH 6 is higher than at pH 8. The poly(acrylamide)-starch graft copolymer based on H-3-starch has a higher flocculation efficiency than polyacrylamide.
ISSN	*0038-9056*

Title	Anaerobic digestion of polyelectrolyte flocculated waste activated sludge
Author(s)	Chu, CP | Lee, DJ | Chang, BV | You, CH | Liao, CS | Tay, JH
Source	CHEMOSPHERE | 53 (7): 757-764 NOV 2003
Times Cited	10
Publication Year	2003
Abstract	This work examined how adding one of three polyelectrolyte flocculants (T3052: cationic, T2000: non-ionic, and T1052: anionic) affected the anaerobic digestion of wastewater sludge. Methane production, floc characteristics (morphology and C-potential) and process parameters (soluble chemical oxygen demands (SCODs) and reductive potentials) were monitored along the digestion tests. The digestion rates of T2000- and T1052-conditioned sludge resembled that for original sludge. The T3052-flocculated sludge generated methane at a higher rate during the first 6 days of digestion than did the original one. In the following stage, the digestion rate of sludge flocculated with T3052 at dosage exceeding 15 g/kg dried solids declined. For example, at 40 days of digestion the methane production amounts for original, 15 g/kg DS flocculated, and 40 g/kg flocculated sludge were of 136, 105, and 85 g/kg DS, respectively. The role of flocculants could change in different stages of digestion. The dosed polymers had no apparent toxicity to the inoculum used. The changes in SCOD, adenosintriphosphate concentrations, oxidative and reductive potential, and zeta-potentials did not correlate with the noted hindered digestion for T3052-conditioned sludge. Microphotographic observation revealed that the floes of T3052-conditioned sludge were not only of a large size, but also were resistant to structural deterioration during digestion. Therefore, mass transfer resistance was proposed to account for the hindered digestion efficiency observed for T3052-conditioned sludge. (C) 2003 Elsevier Ltd. All rights reserved.
ISSN	*0045-6535*

Title	Studies on flocculation of Egyptian kaolin suspensions by anionic polyacrylamide in the presence of surfactants
Author(s)	Abd El-Rahiem, FH
Source	TENSIDE SURFACTANTS DETERGENTS | 42 (2): 88-94 MAR-APR 2005
Times Cited	0
Publication Year	2005
Abstract	This papers reports the results of studies on flocculation of kaolin suspension by anionic polyacrylamide (PAM-A) flocculants in the presence of surfactant. They were namely anionic Sodium Dodecyl Sulphate (SDS), cationic Cetyl Trimethyl Ammonium Bromide (CTAB) and a non-ionic surfactant of polyoxyethylenic ether with trade name Triton (TX 100). The adsorption of PAM-A for fresh kaolin as well as surfactant pretreatment kaolin corresponding to about 50% coverage on the surface. Pretreatment of kaolin with all three surfactants form bigger flocs with PAM-A. The highest settling rate is obtained by flocculation the SDS-pretreatment kaolin. The influence of simultaneous addition of PAM-A and surfactants from their mixtures on flocculation is dependent on the nature of mutual interaction of PAM-A and surfactant. Simultaneous addition of PAM-A with either SDS or TX 100 increases settling rate, whereas it decreases when added from its mixture with CTAB. On the other hand, the optimum dose of PAM-A and 0.2 g/ kg SDS-pretreatment preconcentrate El-Tih Egyptian kaolin is applied. The flocculation product a kaolin concentrate of minimum TiO2 content (0.27%) and the degree of whiteness (93.5) is obtained from TiO2 content (1.52%) and the degree of whiteness (63) of the original.
ISSN	*0932-3414*

Title	Influence of surfactants on flocculation and dewatering of kaolin suspensions by cationic polyacrylamide (PAM-C) flocculant
Author(s)	Besra, L | Sengupta, DK | Roy, SK | Ay, P
Source	SEPARATION AND PURIFICATION TECHNOLOGY | 30 (3): 251-264 MAR 1 2003
Times Cited	10
Publication Year	2003
Abstract	This paper reports the result of studies on flocculation and dewatering of kaolin suspensions by cationic polyacrylamide (PAM-C) flocculants in presence of surfactants. The surfactants used were namely anionic sodium dodecyl sulphate (SDS), cationic cetyl trimethyl ammonium bromide (CTAB) and non-ionic TritonX 100, which is a polyoxyethylenic ether compound. The unflocculated kaolin has a very slow settling rate of about 0.03 cm/s and can be improved by more than ten times using PAM-C as a flocculating agent. PAM-C adsorbs on kaolin primarily through electrostatic attraction and the flocculation is governed mainly by charge neutralisation and bridging. Partial pre-coating of kaolin with all three surfactants leads to both physical adsorption as well as chemisorption at PAM-C. Pretreating the kaolin with surfactants can further increase or decrease settling rate depending on the type of surfactant used. However, the flocs thus formed shows better filtration and dewatering behaviour estimated in terms of reduction in specific resistance to filtration (SRF) and cake moisture. The minimum SRF occurs under conditions corresponding to far lower adsorption of PAM-C than that for best flocculation. Simultaneous addition of PAM-C and each of the three surfactants decreases settling rate and sediment volume markedly and does not reduce SRF any further but they all reduce cake moisture substantially. The reduction in cake moisture for different PAM-C: surfactant mixture is in the following order: PAM-C: SDS (1: 1) > PAM-C: TX 100(1: 1) > PAM-C: CTAB(1: 1). Low cake moisture in comparison with PAM-C alone, may be a result of reduction in entrapment of excess water in the smaller flocs formed by simultaneous addition with surfactants and to some extent due to hydrophobicity caused by adsorption of surfactants. (C) 2002 Elsevier Science B.V. All rights reserved.
ISSN	*1383-5866*

Title	Agglomerate flotation of fine oxide particles
Author(s)	Sadowski, Z | Polowczyk, I
Source	INTERNATIONAL JOURNAL OF MINERAL PROCESSING | 74 (1-4): 85-90 NOV 19 2004
Times Cited	4
Publication Year	2004
Abstract	This paper presents experimental data on using both flocculants and surfactants for aggregation and flotation of fines. The work has been performed with water suspensions of two oxides, ZnO and MgO. Conventional flotation collectors (sodium oleate (SOL), sodium dodecyl sulphate (SDS) and cetyltrimethylammoniurn bromide (CTAB)) were used. Commercial high molecular weight polymers (MAGNAFLOC 1440, MAGNAFLOC E-10 and MAGNAFLOC 3 5 1) were the flocculants used. Adsorption isotherms of surfactants adsorbing onto the two oxides were determined at natural pH. Adsorption density was the greatest with sodium oleate. Zeta potential measurements indicated that addition of the cationic surfactant caused a positive increase of the zeta potential. The positive zeta potential of MgO decreased with increased anionic surfactant concentration. A decrease in stability indicated that the ZnO suspension pretreated with anionic surfactants caused the formation of larger floes when the cationic flocculant (MAGNAFLOC 1440) was added. Similar observations were obtained for a MgO suspension pretreated with anionic surfactants and reacted with the nonionic flocculant, MAGNAFLOC 351. The negative charged collector (SDS) appears to be good collector for ZnO floc flotation when hydrophobic floes are created by the combination of SDS and either cationic or nonionic flocculants. (C) 2003 Elsevier B.V. All tights reserved.
ISSN	*0301-7516*

Title	Novel biodegradable flocculants based on polysaccharides
Author(s)	Singh, RP | Tripathy, T | Karmakar, GP | Rath, SK | Karmakar, NC | Pandey, SR | Kannan, K | Jain, SK | Lan, NT
Source	CURRENT SCIENCE | 78 (7): 798-803 APR 10 2000
Times Cited	45
Publication Year	2000
Abstract	The waste water and industrial effluent-treatment requires both inorganic and organic flocculants. Among the organic type, polymeric flocculants (synthetic as well as natural) are preferred because of their low dosage, easy handling, inertness to pH changes, production of large cohesive flocs and versatile tailorability, The concern for environmental and ecological issues warrants the use of the biodegradable flocculant in waste water and industrial effluent treatments. Natural polysaccharides such as starch, gums, glues, alginate, etc. function as bridging flocculants. This could be accounted for by the purification and chemical modification, It has been established that by grafting polyacrylamide branches on polysaccharides, the dangling grafted chains have easy approachability to the contaminants. Among the grafted guar gum, xanthan gum, carboxy methyl cellulose and starch, grafted starch flocculates better. Starch consists of amylose (a low molecular weight linear polymer) and amylopectin (a high molecular weight, branched polymer). The grafted amylopectin is found to be a suitable flocculant for various kinds of industrial effluents, Here wt summarize the various investigations carried out using flocculants based on polysaccharides.
ISSN	*0011-3891*

Title	Flocculation-coagulation behaviour sudy of tailings of a fluorspar mineral processing plant
Author(s)	Dzioba, BR | Diaz, AA | Menendez-Aguado, JM
Source	REVISTA DE METALURGIA | 40 (4): 312-316 JUL-AUG 2004
Times Cited	0
Publication Year	2004
Abstract	The objective of this work is to carry out a selection study of the most adequate flocculation-coagulation reagents to reduce the solids content in the overflow from the settling lagoons of a fluorspar processing plant. This overflow has 1% solids content, clarified from a feed of 25% solids in the settling lagoons, and is pilled into a river. The importance of this work is enhanced by the fact that the plant is located in a low water resources area, and that eventually the water from the river is used for agriculture or even human consumption. It is relevant to find a working methodology which allow to improve the efficiency of the clarifying process and minimize the environmental impact. Regarding the research methodology, a series of trials were made at natural pH and 25 degreesC to evaluate the action of those reagents which previously probed to be more efficient in this case: as flocculants were tried BOZEFLOC C 65, SEPARAN AP 273 P, SEPARAN MG 200, and as coagulant aluminium sulfate. The possible interaction of flocculation-coagulation processes was also studied, to obtain optimum result. As analysis criterium the total flocculation time was used, and as all essays were made in 100 ml probes, it corresponds with flocculation rate. As result of this study it can be concluded that the most efficient floculant was SEPARAN MG 200, at 4 g/T, with the observation that an excess of aluminium sulfate gets down the efficiency.
ISSN	*0034-8570*